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Dextran Metabolism Following Infusion of 7.5% NaCl/6% Dextran-70 to Euvolemic and Hemorrhaged Rabbits

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ABSTRACT

Dubick, M.A., B.A. Ryan, J.J. Summary, and C.E. Wade: Dextran metabolism following infusion of 7 5% NaCl/6% dextran-70 to euvolemic and hemorrhaged rabbits Drug Dev Res. 25:29–38, 1992

Dextran metabolism was evaluated in euvolemic and hemorrhaged rabbits following administration of a 7 5%NaCl/6% Dextran-70 (HSD) solution Control rabbits and those bled 8 ml/kg body weight were infused i v with 4 ml/kg of HSD or HSD containing ^{14}C-Dextran-70 Blood samples were withdrawn prior to and at times up to 96 hr after HSD infusion Peak serum dextran concentrations were about 29% higher in hemorrhaged rabbits than in controls, yet serum dextran $t_{1/2}$ was similar in both groups Molecular weight (MW) distribution of dextran in serum showed a slight shift toward a MW >70,000, consistent with excretion of lower MW forms in the urine. After 96 hr concentrations of ^{14}C-Dextran were 20-fold higher in liver from both groups of rabbits, in comparison to spleen, lung, and kidney In addition, dextranase activity in liver was markedly higher than in the other tissues assayed These studies indicate that dextran infused as HSD does not associate with any protein fractions, is found only in low concentrations in tissue, and has a serum half-life adequate to serve as a useful plasma volume expander

Key words: hypertonic resuscitation, Dextran-70, hemorrhage, rabbits, dextranase

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INTRODUCTION

Recent years have seen renewed interest in the use of hypertonic-hyperoncotic solutions to treat hemorrhagic shock [Kramer et al , 1989, Dubick et al., 1989, Wade et al., 1989] Studies in experimental animals have shown small volume infusion of a 7 5% hypertonic saline/6% Dextran-70 (HSD) solution to be effective in restoring cardiovascular and renal function, and tissue blood flow, thereby improving survival following potentially lethal hemorrhage [Dubick et al., 1989; Wade et al., 1989] In human field trials, HSD has also been reported to improve survival of trauma victims [Holcroft et al., 1989] Currently, HSD is being evaluated in Phase III clinical trials at 4 ml/kg body weight, a lower dose than that employed clinically for either Dextran 40 or 70 in physiological saline [Thoren, 1980].

An essential part of the new drug application for HSD filed with the US Food and Drug Administration (FDA), are data associated with its pharmacology. Although the metabolic aspects of previous clinical dextrans have been reported [Hammarsten et al., 1953; Hunt, 1971, Gruber, 1969], FDA guidelines dictate that since HSD is a combination drug, its unique properties must be addressed In addition, information is lacking pertaining to dextran metabolism under conditions of hypovolemia Therefore, the present study investigates aspects of the tissue distribution and metabolism of HSD following its administration to both euvolemic and hemorrhaged rabbits

MATERIALS AND METHODS

Animals and Treatment

Adult, female New Zealand white rabbits (Elkhorn Rabbitry, Watsonville, CA) weighing 2 5 to 3 5 kg, were randomly assigned to either the hemorrhage (n = 10) or control (n = 8) group Rabbits were catheterized via the middle ear artery and in the hemorrhaged group, bled 8 ml/kg body weight over a 15-min period to mimic a moderate hemorrhage This represents about 11% of blood volume and was selected since our preliminary observations indicated that rabbits are relatively intolerant to hemorrhage After a 30-min stabilization period, rabbits in both groups were infused i v with 4 ml/kg body weight with HSD (Lot No . NC 54845) (AB Pharmacia, Uppsala, Sweden) without or with 5 μCi/kg carboxyl-^{14}C-Dextran-70 (Lot No 2275-289, sp act 0 8 mCi/g), DuPont-New England Nuclear, Boston, MA) Blood samples (6 ml) were withdrawn prior to and 0 17, 0 5, 1, 2 4, 6, 24, 48, 72, and 96 hr after the HSD infusion In experiments where no radioactive dextran was infused, a blood sample, was also drawn 7 days after HSD infusion After each blood sample, an equivalent volume of saline was infused back into the animal to help maintain plasma volume During the experimental period, rabbits were individually house in metabolic cages Urine samples collected over 24 hr were centrifuged at 3,000*g* for 10 min and frozen with serum samples at −20°C until assayed

Dextran Measurements

Dextran concentrations in serum and urine were determined by the anthrone reaction following precipitation of serum with 10% trichloloacetic acid (TCA) and oxidation of endogenous glucose with glucose oxidase [Weet et al , 1976] In addition hemoglobin and hematocrit concentrations were determined as part of CBC measurements and protein concentrations were determined by the Biuret assay on a Cobas Fara II Autoanalyzer (Roche Analytical Instruments, Belleville, NJ). Changes in hemoglobin, hematocrit or protein concentrations were used to estimate plasma volume expansion as previously described [Dubick et al , 1989, Halvorsen et al , 1989] In the experiments employing ^{14}C-dextran, an aliquot of serum and deproteinized serum was counted for radioactivity by liquid scintillation Data were expressed as dpm/ml serum or dpm/mg dextran

Gel Filtration

To quantitate the molecular weight distribution of the dextran fractions following HSD infusion, protein-free serum aliquots were applied to a 0.9 × 87 cm column of Sephadex 200/100 [Nilsson and Nilsson, 1974] equilibrated with 0.3% NaCl and eluted with the same solution, as previously described [Dubick et al., 1989].

Tissue Distribution

The tissue distribution of ^{14}C-dextran in liver, lung, kidney, and spleen was determined at the end of the 96 hr experimental period. These tissues have been shown previously as primarily involved in dextran metabolism [Gruber, 1969]. Tissues were oxidized in a Packard Tricarb Oxidizer (Packard Instruments, Downer's Grove, IL) and data expressed as dpm/g tissue. In other experiments, the uptake and binding of ^{14}C-Dextran-70 to crude membrane fractions were determined in vitro with liver. Crude liver membrane fractions were prepared according to the method of Dangott et al. [1986], and incubated with the ^{14}C-dextran in the absence or presence of a 100-fold excess of cold Dextran 70, for 0, 5, 10, 15, and 30 min. Time 0 samples were collected immediately after addition of ^{14}C-Dextran-70. After centrifuging in a microfuge, the resultant pellet was washed four times with saline. The final pellet was resuspended in the membrane buffer and an aliquot counted for radioactivity by liquid scintillation.

Dextranase Activity

To further evaluate dextran metabolism, dextranase (E.C.3.2.1.11) activity in liver, lung, kidney, and spleen was determined at the end of the 96-hr experimental period according to the method of Janson and Porath, as described in the *Worthington Manual, 1988* (Worthington, Freehold, NJ). Activity was expressed as mU/mg protein.

Statistical Analysis

The radioisotope dilution technique was employed to evaluate pharmacokinetic parameters of dextran during the first 24 hr following HSD infusion in both euvolemic and hemorrhaged rabbits. The best-fit for ^{14}C-Dextran-70 disappearance curves were plotted on a semilog scale and analyzed by least squares non-linear regression [Remington and Schork, 1970] to determine half-life. A BMDP non-linear regression program was employed for kinetic analysis [Ralston et al., 1979]. The best fit of the data was described by a 1-compartment model defined by the equation $Y = Ae^{-kt}$, where Y is the concentration of drug at time t, A is the concentration of drug administered at time 0, k is the rate constant of elimination, and t is time. Statistical comparison of the kinetic parameters derived, dextranase activity, and tissue distribution between the two groups was by Student's t-test with $P<0.05$ considered significant [Remington and Schork, 1970]. Analysis of variance was used to analyze liver membrane binding and liver uptake of dextran with time as the independent variable [Remington and Schork, 1970].

RESULTS

Dextran Concentrations and Clearance

In the present study, rabbits were weight matched so that similar amounts of dextran as HSD were administered to both the euvolemic control (12.4±0.6 ml) and hemorrhaged (13.0±0.4 ml) groups. Nevertheless, serum dextran concentrations, measured 10 min following HSD infusion, were about 20% higher in hemorrhaged rabbits than in controls (Table 1). Plasma volume expansion by HSD in hemorrhaged rabbits averaged 5–10% greater than in euvolemic controls. Therefore, if this difference in hemodilution between groups was ac-

TABLE 1. Effect of HSD Administration on Dextran Concentrations and Pharmacokinetic Parameters in Serum From Euvolemic and Hemorrhaged Rabbits[†]

	Euvolemic	Hemorrhaged
Dextran concentration[a] (mg/dl)	474 ± 28 (8	572 ± 31 (10)*
Half-life (hr)	. 0 ± 0 5 (8)	7 4 ± 0 4 (10)
Plasma clearance (ml/hr)	13 3 ± 1 6 (8)	11 0 ± 1 1 (10)

[†]Data expressed as mean ±SE (n)
[a]Highest concentration measured at 10 min after HSD infusion
*$P<0$ 05 different from euvolemic control

counted for, serum dextran concentrations would be 29% higher in the hemorrhaged group than controls

In both the euvolemic and hemorrhaged rabbits, dextran concentrations in serum were highest at the initial sampling time. Dextran concentrations decreased over time and were generally undetectable after 48 to 72 hr Based on the best-fit regression model, a plot of serum dextran concentrations vs. time depicted that dextran $t_{1/2}$ in serum was 7 0 and 7 4 hr in control and hemorrhaged rabbits, respectively (Fig 1, Table 1) In addition, calculation of serum clearance from the dose of HSD administered and the area under the serum concentration vs time curve, revealed that clearance appeared to be about 21% lower in the hemorrhaged rabbits than their euvolemic counterparts However, the differences were not statistically significant (Table 1) Clearance of the radiolabeled Dextran-70 paralleled the disappearance of dextran in HSD (data not shown) As shown in Figure 2, ^{14}C-Dextran-70 contained lower molecular weight components than the Dextran-70 in HSD Therefore, only the cold dextran serum data were used to evaluate dextran turnover from serum in these studies However, ^{14}C-Dextran-70 metabolism indicated that dextran was not bound to serum proteins

Gel filtration chromatography was employed to detect changes in the molecular weight distribution of the administered dextran for up to 6 hr after infusion in serum and 24 hr in urine At each time point assayed, no differences in molecular weight distribution were observed in serum between hemorrhaged or euvolemic rabbits In comparison to the native ^{14}C-Dextran-70 infused, the molecular weight profile of ^{14}C-Dextran at 6 hr post-infusion showed the typical slight shift to the left (Fig 2A) In urine from both groups of rabbits, approximately 25 to 30% of the administered dose of ^{14}C-Dextran-70 was excreted in the first 24 hr In addition, at each time point assayed no appreciable differences in the molecular weight distribution of ^{14}C-Dextran was observed between euvolemic and hemorrhaged rabbits (Fig 2B) At 6 and 24 hr the major urinary ^{14}C-Dextran peak corresponded to molecular weights less than 40,000

Tissue Distribution

At the end of the 96-hr experimental period, concentrations of ^{14}C-Dextran-70 were determined in liver, kidney, spleen, and lung from both groups of rabbits As shown in Figure 3A, concentrations of labeled dextran, expressed as dpm/g tissue, were similar in lung, spleen and kidney, whereas they were about 20-fold higher in liver In liver these concentrations of dextran were approximately 39 μg/g or < 10% of the infused dose Again no significant differences were observed between the 2 groups of rabbits (Fig 3A)

Since ^{14}C-Dextran concentrations were markedly higher in liver, other experiments examined the binding of dextran to liver as well as its uptake ^{14}C-Dextran-70 bound rapidly to crude liver membrane preparations, but the degree of binding at each time point was not significantly higher than the background bound-to-free ratio (B/F) For example, the B/F ratio at 30 min was 6 0±1 3 $\times 10^{-4}$/mg protein compared with 3 7±0 6 $\times 10^{-4}$/mg protein at time 0 In addition, the binding could not be displaced by over 100-fold excess cold Dextran-70, further suggesting that the binding was non-specific in nature

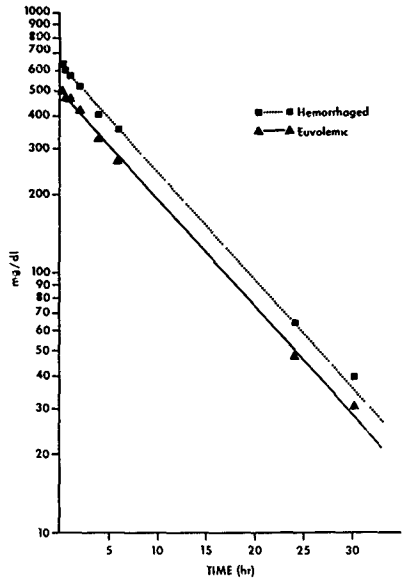

Fig 1 Dextran clearance from serum of euvolemic (solid line) and hemorrhaged rabbits (dashed line) Data represent mean ± SE of eight euvolemic and ten hemorrhaged rabbits The SE are contained within the size of the symbols Lines are derived from linear regression kinetic analysis of the dextran concentrations measured Other details of the parameters that define the line are presented in the methods section

Dextranase Activity

Additional experiments determined the dextranase activity in liver and the other tissues assayed The highest dextranase specific activity, expressed as mU/mg protein, was found in liver and kidney (Fig 3B). Of the tissues assayed, the lowest activity was found in lung Dextranase activity in liver and spleen from hemorrhaged rabbits were about 20% higher than in tissue from control rabbits, but the differences were not statistically significant (Fig 3B) In contrast lung dextranase activity was 19% lower in hemorrhaged rabbits than in controls (Fig 3B)

DISCUSSION

In the present study, dextran concentrations in serum peaked early and were significantly higher in the hemorrhaged rabbits in comparison to the euvolemic control These results are consistent with our previous observation following administration of HSD at a dose of 4 ml/kg to hemorrhaged and euvolemic swine [Dubick et al , 1989] and probably reflect differences in blood volume due to the hemorrhage In addition, we observed the typical shift to higher molecular weight dextran components in serum over a 6-hr period, consistent with previous reports that low molecular weight components are rapidly excreted by the kidney [Arturson

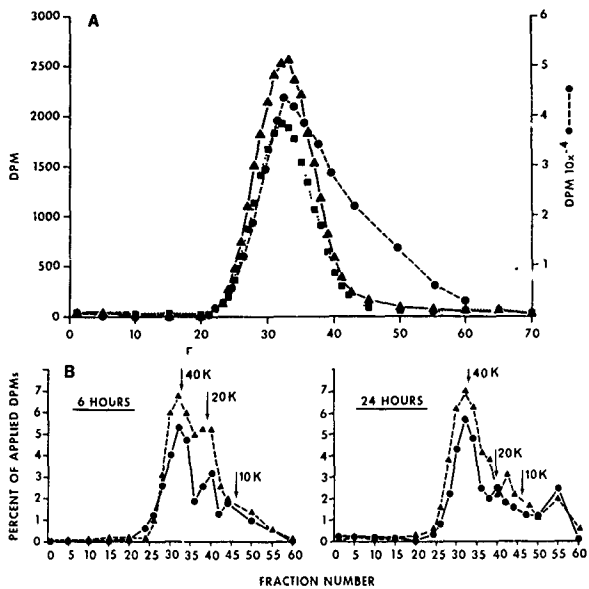

Fig 2 **A:** Molecular weight distribution of ^{14}C-Dextran-70 in serum from euvolemic and hemorrhaged rabbits Profiles at 6 hr post-infusion are compared with initial profiles of the native ^{14}C-Dextran-70 (●– – –●) Hemorrhaged rabbit (▲—▲), euvolemic rabbit, (■– – –■) **B:** Molecular weight distribution of ^{14}C-Dextran in urine from euvolemic and hemorrhaged rabbits Data are expressed as a percentage of DPMs applied to the column and represent data averaged from two rabbits/group at each time period Euvolemic (●—●), hemorrhaged (▲– – –▲)

and Wallenius, 1964a] while glomerular filtration of dextrans with molecular weights >50–60,000 is very low and dextrans are neither reabsorbed nor secreted by the renal tubules [Arturson and Wallenius, 1964b, Arturson et al , 1966, Leypoldt et al , 1987]

In addition, the lack of small molecular weight components in serum during this time period agrees with reports that dextranases do not exist in serum [Rosenfeld and Lukomskaya, 1957] Thus, it appears that, at least in the 6-hr period monitored, the dextran detected in serum is of sufficient molecular weight to serve as a plasma volume expander Despite the difference in serum concentrations, dextran turnover from serum was not significantly different between control and resuscitated hemorrhaged rabbits. In the first 24 hr, the change in serum dextran concentrations over time was best described by a one-compartment model and is consistent with the observation that dextrans distribute rapidly following i v administration [Gruber, 1969] These data indicated that the serum $t_{1/2}$ of Dextran-70, administered as HSD, was about 7 hr and is consistent with $t_{1/2}$ of 6 2 hr following administration of Dextran-60 in young children [Arturson et al , 1966] However, the value is lower that the >12 hr reported in normal adults following infusion of dextrans with molecular weights of 55,000 to 69,000 [Arturson and Wallenius, 1964a] It should be noted that HSD differs from early clinical

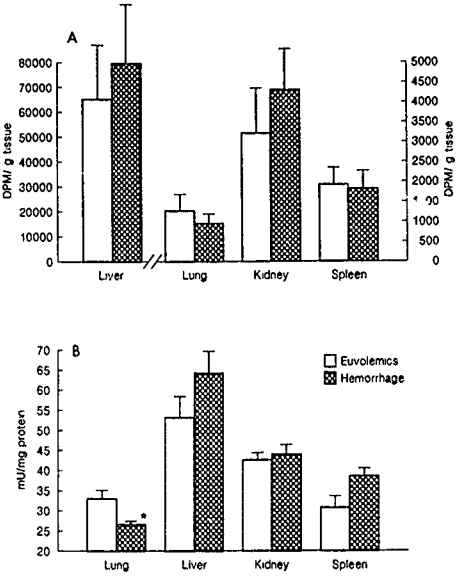

Fig 3 **A:** Tissue distribution of ^{14}C-Dextran in euvolemic (n = 5) and hemorrhaged (n = 7) rabbits after 96 hr experimental period Data expressed as mean ±SE of DPM/g tissue Euvolemic, open bar, hemorrhaged, hatched bar **B:** Tissue dextranase activity in euvolemic (n = 7) and hemorrhaged (n = 8) rabbits after 96 hr experimental period Data expressed as mean ±SE of mU/mg protein Euvolemic, open bar, hemorrhaged, hatched bar *P<0 05 from euvolemic control

Dextran-70 in that its molecular weight range is narrower, ranging from 20,000 to 100,000 compared to 25,000 to 200,000, and this may account, at least in part, for the differences in serum $t_{1/2}$ observed

Although the $t_{1/2}$ of dextran in serum was similar in both groups of rabbits, it appeared that dextran clearance was about 21% slower in hemorrhaged animals than their euvolemic counterparts As a plasma volume expander, infusion of dextran causes a hemodilution that is a function of the dextran dose and time after infusion Previous reports discussing pharmacokinetics of plasma volume expander [Mishler, 1984, Klotz and Kroemer, 1987] have not dealt with differential volume expansion as observed in euvolemic vs hemorrhaged animals nor have they discussed possible difficulty in data interpretation as volume expansion changes over time Nevertheless, it is clear that conventional pharmacokinetic analysis cannot be strictly applied to plasma volume expanders that are heterogeneous in molecular size, such as dextrans and hydroxyethyl starch [Klotz and Kroemer, 1987, Yacobi et al , 1982]

As previously mentioned, studies with other clinical dextrans indicate that initially, dextrans are primarily cleared through the kidney [Arturson and Wallenius, 1964b, Arturson et al , 1964] This reflects its major route of metabolism in the first hours following its infusion In the present study, approximately 25 to 30% of the administered dextran was excreted in urine in 24 hr, slightly less than 31–47% previously reported for Dextran-60 and

-70 [Thoren, 1980, Arturson and Wallenius, 1964b, Howard et al , 1956, Harrison, 1954] Thus, if renal function is not impaired by an induced hypovolemic state or is corrected following resuscitation [Dubick et al , 1989] it seems reasonable to assume that dextran turnover would be similar in both groups of rabbits It should be mentioned that some authors reported that dextran clearance followed a biphasic pattern [Emmrich et al , 1977] In these situations it appeared that the first phase mainly represents renal clearance, while the second phase presumably denotes dextran distribution and metabolism in tissues [Gray, 1953] It is reported that dextran metabolism is a slow process [Gruber, 1969] and is insignificant with respect to the rate of renal clearance However, this metabolism plays a more important role after low molecular weight components of dextran are excreted Therefore, in the context of HSD as a resuscitation fluid for use in the field prior to transport to the hospital, this second phase of dextran clearance can be ignored

The present data also found that 96 hr after HSD administration, concentrations of ^{14}C-Dextran were significantly higher in liver than in kidney, spleen, or lung. In vitro, ^{14}C-Dextran uptake by liver was non-specific in nature and was not associated with tissue protein or membrane structures Previous studies in experimental animals reported that dextran accumulated in liver, kidney, and spleen [Persson, 1952, Swedin and Aberg, 1952, Linder, 1971] and that dextran concentrations in liver declined rapidly when plasma concentrations fell to undetectable levels Although some storage of dextran in tissues has been observed by us and others, it does not appear to be associated with any toxic effects and is completely catabolized over time [Gruber, 1969]

Since Gray [1953] first suggested that dextran could be metabolized by mammals, and its components incorporated into the body's carbon pool, a number of studies have shown the presence of dextranases in mammalian tissue, including human [Rosenfeld and Lukomskaya, 1957; Ammon, 1963]. In the present study, dextranase activity was detected in all tissues assayed, with the highest specific activity in liver, followed by kidney, spleen, and lung Although dextranase activity in lung from hemorrhaged rabbits was significantly lower than in euvolemic controls, overall, dextranase activity did not appear to be significantly affected by hemorrhage In agreement with previous reports [Halmagyi, 1979], tissue dextranase also did not appear to contribute significantly to the observed rate of dextran clearance from serum. However, in the overall understanding of dextran metabolism in mammalian tissue, future studies on the relationship between dextranase activity and rates of tissue dextran uptake may be warranted

Thus, in conclusion, the data from the present study indicate that HSD resuscitation of hypovolemia does not alter dextran metabolism at the tissue level in comparison to normal animals Most significantly, given as a single bolus of 4 ml/kg, it has a serum $t_{1/2}$ suitable for its use as a pre-emergency room volume expander

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